

COMPARISON BETWEEN SPERM  
AGGLUTINATING ACTIVITY IN SERUM AND  
CERVICAL MUCUS OF INFERTILE WOMEN

THESIS  
FOR  
MASTER OF SURGERY  
(OBSTETRICS AND GYNAECOLOGY)



BUNDELKHAND UNIVERSITY  
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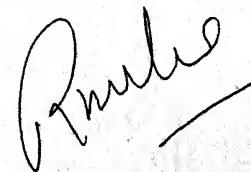
A handwritten signature in black ink, appearing to read "Archana Srivastava".

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C E R T I F I C A T E

This is to certify that the work entitled "Comparison between sperm agglutinating activity in serum and cervical mucus of infertile women", which is being submitted as a thesis for M.S. (Obstetrics and Gynaecology), has been carried out by Dr. Archana Srivastava in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.

The candidate has fulfilled the necessary stay in the department as per University regulations.



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This is to certify that the work entitled "Comparison between sperm agglutinating activity in serum and cervical mucus of infertile women", which is being submitted as a thesis for M.S. (Obstetrics and Gynaecology) by Dr. Archana Srivastava has been carried out under my direct supervision and guidance in the Department of Obstetrics and Gynaecology. The techniques and methods described were undertaken by the candidate herself and the observations recorded have been periodically checked and verified by me.

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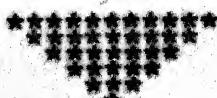
Lastly, I am thankful to Mr. K.M. Thomas for painstaking neatness in putting this work into black and white.

Archana Srivastava

( ARCHANA SRIVASTAVA )

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INTRODUCTION

## INTRODUCTION

The age old problem of the barren marriage has always engaged the interest of the physician. However with the advance of civilization it has become more than eternal medical problem, a problem which closely affects the welfare of the society because reproduction is an essential aspect of marriage.

The desire for children by the normal woman is stronger than self-interest in beauty and figure, stronger than the claims of career, in the male it is less intense. Childlessness is generally a tragedy to the married women, and can be a cause of marital upset as well as of personal unhappiness and ill health. Moreover sterility ranks high among the causes of deep unhappiness in marriages because childless union lacks the strong cementing desire for the common good of the family.

Since time immemorial, the wife has always been blamed for infertility and because of psychological upbearing in our society, the women usually assumes the responsibility for failure to produce a child.

Failure to find sperms in coital test by Huhner in 1913 raised the possibility that the husband could also be responsible for infertility. Since then, the available statistics from various infertility clinics show that the husband is responsible for infertility, totally or partially, in 40-50 percent of infertile couples. This realisation also gives an idea of the magnitude of the problem.

So it is important to remember that infertility is a disorder of complex nature where both the partners are equally responsible.

Unexplained failure in infertile cases, with absence of any evidence of pathology, left the clinician in a blind alley. Hence researches were done in this aspect of field, to evaluate the cause for infertility and to provide comprehensive management with better results.

At the turn of this century, it was observed that spermatozoa from one animal species may provoke an immune reaction not only in heterologous species, but also in male or female of the same species (Landsteiner, 1899; Metchnikoff, 1900). Antibodies demonstrated in such experiments are termed hetero-auto and iso antibodies respectively (Voisin et al., 1974).

Over a past eighty years a considerable amount of data has accumulated from extensive investigations in reproductive immunology. The main ventures in this field have been to disclose the immunological reactions as a cause of infertility and conversely to investigate the possibility of active fertility regulation by inducing immune reactions against reproductive antigens i.e. the development of contraceptive vaccine. These objectives have been approached by investigating the association between infertility and spontaneously developed immune reactions against antigens in the male or female reproductive tract "Nature's own experiments" - and secondly by experimental studies on the effect on fertility of active immunization with such antigens.

Immunization of female animals with spermatozoa or testes from homologous males has resulted in significant reduction of fertility in an extensive number of investigations (Katser, 1959; Tyler, 1961; Henge, 1970; Behrman, 1975). Today such experiments seem to be rather primitive and unethical, since immunization with crude spermatozoa material implies an obvious risk of developing

immunological disease as a cross-reacting antibodies, immune-complex and allergic reaction (Tung, 1976).

Therefore in human reproductive immunology, new knowledge has been gained by studying "spontaneously" developed immune reaction against reproductive antigens.

Exposure to foreign antigens may lead to one or several type of immune reactions which are classified as follows : (i) Anaphylatic, (ii) cytotoxic, (iii) complex-mediated, (iv) cell mediated, (iv) stimulatory hypersensitivity.

The investigation of immune reactions in women against sperm antigens have concentrated mainly on type II reactions. These reactions appear to be due to IgE antibodies directed against a non-spermatozoa glycoprotein in seminal plasma.

A cytotoxic immune reaction against spermatozoa was first observed in women by Meeker in 1922. He found sperm agglutination and immobilizing activity in serum from a woman from couples with so called unexplained infertility. However, it was not until 1964 that more extensive investigation appeared on the occurrence of sperm antibodies in

fertile and infertile women. In these initial studies, Franklin-Dukes (1964 a, b) using a micro-agglutination technique, observed frequently in women from couples with unexplained infertility.

In systemically immunized experimental animals, sperm antibodies have been demonstrated in all segments of the female genital tract, i.e. the vagina, cervix, uterus, fallopian tube and in the follicular fluid (By Menge, 1970; Kille & Goldberg, 1979).

Increased phagocytosis of spermatozoa in uterus (Sokolovskaya & Reshetnikova, 1969), the fertility reducing effect of sperm antibodies in immunized female has been ascribed to inhibited penetration of spermatozoa in the genital tract (Metz & Amnika, 1970; Menge, 1971 a), inhibited fertilization of the ovum (Menge, 1970; Menge, 1971 b; Metz et al, 1972; Munoz & Metz, 1978) and early embryo mortality (Menge, 1968, 1969 a, 1970 & Menge et al, 1974).

These animal experiments have therefore not only demonstrated that iso-immunization with spermatozoa may reduce fertility, but have also indicated some of the possible mechanism behind this effect.

Despite the fact that spermatozoa were demonstrated as antigenic over 90 years ago, controversy still concerning what effect, if any, sperm antibodies have on fertility.

In view of this the present study was undertaken with aims -

1. To evaluate the incidence of sperm agglutinating antibodies in serum of women having unexplained infertility.
2. To evaluate the incidence of sperm agglutinating antibodies in cervical mucus of women having unexplained infertility.
3. To compare the significance of sperm agglutinating antibodies in serum and cervical mucus in women having unexplained infertility.

\*\*\*\*\*

**REVIEW OF LITERATURE**

## REVIEW OF LITERATURE

Immunologic reaction is the principle means of defense against antigen. A fundamental characteristic of an animal immune system is that it does not react against its own body constituents under normal circumstances. There exists a mechanism that enables the cells of the immune system to recognize what is 'self' and what is 'foreign'. Attempts to explain the absence of reactions against self go back to the early days of immunology.

Infertility of immunologic origin is an attractive working hypothesis that has not yet been thoroughly corroborated.

Infertility of immunologic origin is due to immunization of man to his own spermatozoa or immunization of the woman to her husband's spermatozoa. It is more appropriate to speak of "diminished fertility" of immunologic origin than to infertility.

### History

Von Leeuwenhook (1679) first observed sperm in semen. He believed that the "animalcules"

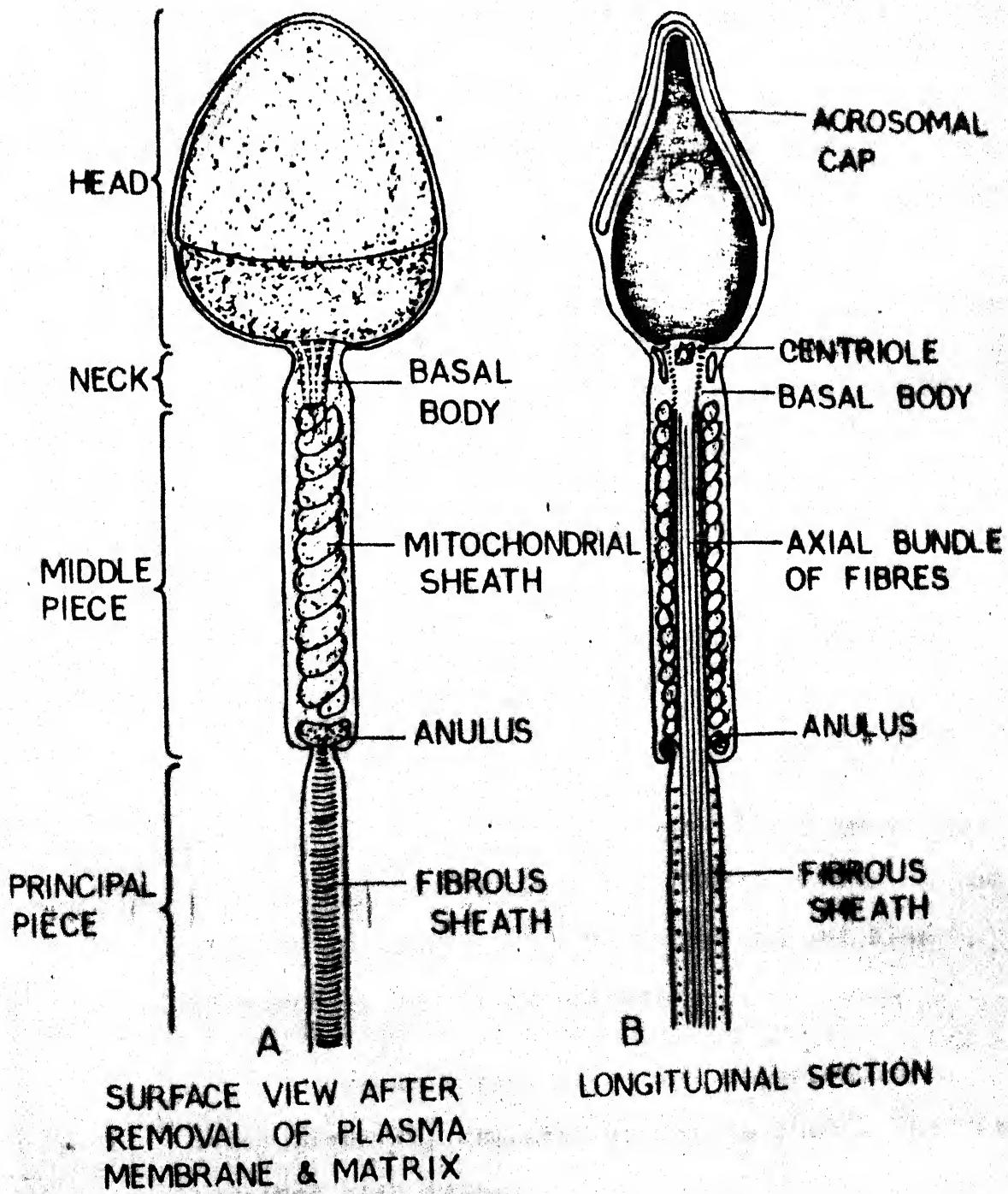
visualised embodied a whole human being in miniature, which developed after contact with vaginal fluid, more than 200 yrs ago until Retzius (1890) performed exhaustive comparative anatomical studies of sperm in numerous species, including man and defined the true nature of cells (Fig. 1).

Antigenicity of sperm demonstrated by Landsteiner, Metchnikoff and Metalnikov (1899) by injecting the sperm or testicular extracts into the experimental animals resulted in antibody formation. Von Moxter (1900) injected rat sperm into the rabbits and obtained antisera which were spermicidal to rat sperm. de Leslie (1901) noted that male mice become sterile for short intervals after receiving injection of antisperm serum obtained in guinea pigs.

Farnum (1901) injected female rabbits intraperitoneally five to eight times with semen or testicular material of dog, bull or man at interval of 2-6 days and concluded that sera of the treated animals contained precipitins which were specific for each antigen.

Pfeiffer (1901) also demonstrated the antigenicity of heterologous mammalian spermatozoa in rabbit.

FIG NO-1  
DIAGRAM TO ILLUSTRATE THE STRUCTURE  
OF THE HUMAN SPERMATOZOOON



Strube (1902) discovered the antigenicity of human semen in rabbit.

Ricketts (1906) has recorded that castrated animals can be immunized successfully with sperm.

Taylor (1908) concluded that the ether soluble fraction of salmon sperm did not induce antibody formation in rabbits, but that injection of whole sperm resulted in an antiserum which was cytotoxic for salmon sperm.

In 1911, Sovini attempted to induce sterility in female rabbits and guinea-pig with sperm injections.

Metalnikov and Strelnikov (1913) placed sperm and testicular grafts with and without enclosure in colloidon sacs in body tissues and recorded antibody production.

Phenomena of agglutination was evolved from Lillie's theory (1912) and it was based upon Enrlich's side chain theory. The identity of the agglutinin substances and fertilizin was also advanced in theory by Lillie.

Venema (1916) cited the experiments in which sterility resulted in female rabbit that were injected with testes.

Dittler (1920) for the first time proposed that sterilization required the use of homologous material from the testes.

Guyer (1922) prepared antisera by injecting fowl repeatedly with rabbit sperm and found such sera not only toxic to rabbit and guinea pig sperm *in vitro* but also induced sterility in male rabbits.

Mc-Cartney (1923) injected rat or human sperm or testes extract into the female rats and observed sterility. Serological examination implied that the infertility was due to the presence of sperm toxins in the vaginal and uterine secretions, since these fluids immobilized and agglutinated sperm.

Kennedy (1924) reported that

- (a) both male and female guinea pig could be sterilized by injecting guinea pig sperm.
- (b) The sperm immobilizing capacity of the antibody serum was more potent in the sensitized male than it was in the immunized female.
- (c) Degenerative changes occurred in the testicles of some of the injected males.
- (d) Autologous injection were most effective in inducing sterility in the male.

Pommerenke (1928) made the following observations -

1. The serum as well as the vaginal secretions of female rabbits injected with rabbit sperm or testicular extracts were toxic for rabbit sperm.
2. After intravenous injections of rabbit sperm or testis extract into the female rabbits, the longevity of sperm deposited in the genital tract during mating was greatly decreased.
3. Infertility for 6 to 25 wk was induced following repeated injection of sperm or testes extract in the female rabbit and injection of salivary gland or ejaculate of vasectomized males had no effect.
4. Sterility caused by sperm of testes injection was not due to an effect on the ovulatory mechanism but if such materials were given during pregnancy, abortion or resorption of fetuses occurred in some cases.
5. Apparently cross reactions occurred between rabbit and rat sperm because serum of rabbits injected with rabbit sperm was toxic for rat sperm and serum of rabbit which received rat sperm was toxic for rat sperm.

6. Repeated intravaginal injections of rabbit sperm into female rabbits led to detectable antigenicity in the serum as well as in the vaginal secretions of these rabbits but Pommerenke was unable to obtain conclusive evidence that sterility could be induced by intravaginal injection of sperm.

In 1926 Landsteiner and Levine demonstrated that sperm cells of humans of appropriate blood type absorbed specifically and almost completely immune antibodies to the A and B antigen of human erythrocytes.

Mudd and Mudd (1929) demonstrated that the sperm of man, guinea pig, bull and rat when injected into rabbits induced antibodies which were species specific. They conclude that mammalian sperm possessed both species and tissue specificity.

In 1937 U.S. Patent number 2,103,240 was awarded to Baskin (1937) for a non-specific spermatoxic vaccine and for the process involved in production of the vaccine.

Henle (1938) conducted the extensive studies on the antigenic nature of mammalian sperm and characterized especially the antigens of bull sperm, heat labile head specific and tail specific antigens

and heat stable antigen common to both head and tail which was species specific were observed.

Eastman, Guttmacher and Stewart (1939) were intrigued by the possibility of inducing sterility in female by means of sperm injection.

Lamoreaux (1940) were demonstrated the high titers of antibodies in laying hens by injecting homologous sperm.

Voisin and Delaunay (1951) demonstrated that aspermatogenesis could be induced by immunologic means. Docten & collaborators (1952) presented the evidence that bovine isoimmune sera containing antibodies for bovine erythrocytes also reacted specifically with bovine sperm.

Meanwhile the work of Wilson (1954) advanced another aspect of male infertility when he demonstrated that agglutination of normal human sperm was produced by the seminal plasma and blood serum of 2 patients whose spermatozoa exhibited auto-agglutination.

This work has been carried further by Weil and associates (1956) and Wilson (1967), presented agglutination due to auto-antibody along with immunologic findings.

Immunization of female animals with spermatozoa of testes from homologous males had resulted in a significant reduction of fertility in an extensive number of investigations (Katsen, 1959; Tyler, 1961; Mengs, 1970; Behrmen, 1975).

### Sperm Antigens

Sperm antigens are present on the surface membrane of human spermatozoa. They are divided into three groups.

Spermatozoa from secretors carry ABO antigen which has been confirmed by several most recent investigations applying absorptive procedures, mixed

agglutination and immunofluorescence techniques (Edward et al, 1964; Boettcher, 1968).

It is not clear whether ABO antigen are coated on to spermatozoa from seminal plasma or primarily integrated into the sperm membrane (Popivanov and Vulchanov, 1969). Antibodies against ABO-antigens do not, however, induce sperm agglutination as detected by the gelatin agglutination technique or by a micro-agglutination test (Fernandes Collaro and Thieres, 1972).

HLA Antigen - The presence of HLA antigens on spermatozoa has been suggested by result from a variety of technique such as cytotoxicity test, absorption procedure, indirect immunofluorescence and immuno-electronmicroscopy by Fellous & Dausset, 1970; Kerek & Afzelius, 1972; Kerek et al, 1973; Halim et al, 1974; Festenstein & Halim, 1977; Festenstein et al, 1978). However, other investigator have failed to demonstrate histocompatibility specificities on human spermatozoa (Law & Bodmer, 1978; Stenbjerje).

2. Sperm coating antigen - Human spermatozoa become coated by antigenic material at their passage through seminal vesicle (Weil, 1967). Li & Behrman (1970) had described the three, so called sperm coating

antigens. The results have suggested that sperm immobilizing antibodies from some infertile women are directed against antigen coated on to spermatozoa from seminal plasma (Isojuna et al, 1968; Isojima et al, 1977).

Ingerslev (1981) concluded from his studies that sperm agglutinating and immobilizing antibodies in human sera do not seem to be directed against sperm coating antigens.

### 3. Organ-specific antigen -

Since sperm antibodies are absorbable with testes homogenate and washed spermatozoa, and do not react with allo-antigens or sperm coating antigens, therefore it seem justifiable to regard these antibodies as organ-specific and intrinsic to spermatozoa. The possibility that bacteria or other micro-organism may induce antibodies in human cross-reacting with spermatozoal surface membrane antigens does not seem to have been investigated.

The antigens reacting with sperm agglutinating and immobilizing antibodies have not yet been isolated or biochemically characterized. However, by microscopical observation of the morphology of sperm agglutinates it is possible to

discriminate between different modes of agglutination which have been interpreted to represent antibodies of different antigen specificity. Thus the antibodies in some sera seems to react selectively with antigens on the head (head to head agglutination) or on the tail (tail to tail agglutination) whereas the picture in other cases is less clear i.e. mixed and tangled agglutination (Ingerslev, 1981).

Sperm antibodies - Sperm antibodies demonstrated by various experiments have been termed hetero (heterologous species), auto (male), and iso-antibodies (female) by Voisin et al in 1974. These antibodies destroy, immobilize or agglutinate the spermatozoa according to their nature (Boettcher et al, Ingerslev, 1979).

Immunoglobulin classes of sperm agglutinating antibodies -

The immunoglobulin class of sperm antibodies in serum and cervical mucus has been investigated by various techniques, based on physico-chemical principles or on immunological affinity binding.

In sera antibodies principally belong to the IgG and IgM class, while IgA antibodies have been observed in very low titer (Boettcher et al, 1971;

Isojima et al, 1972; Friberg, 1974 b; Ingerslev, 1979; Ingerslev et al, 1979).

While the cervical mucus contain predominance of IgA antibodies, IgG in very little amount while IgM almost absent in cervical mucus.

Unique physico-chemical properties of IgA in cervical mucus are -

IgA usually trimeric or polymeric. Containing low molecular weight, secretory component having a total molecular weight of approximately 390,000 and a sedimentation coefficient of 11 s, for 80-90% of IgA secretions.

While IgA in human sera is principally monomeric, with a sedimentation coefficient of 7 s.

11 s-IgA of cervical mucus having slower diffusion rate than 7 s-IgA of serum.

Cytotoxic antibody immobilize the sperm in cervical mucus was found to be IgG globulin in nature. It cause no morphological disturbance in spermatozoa (Parish et al, 1967).

Sperm agglutination in human sera

Meeker in 1922 first observed cytotoxic immune reaction against spermatozoa in women. He found sperm agglutinating and immobilizing activity in serum from two women from couples with so-called unexplained infertility.

Experimental immunization of women with human spermatozoa has been performed in a few investigations leading to the development of sperm antibodies and apparently reduced fertility (Baskin, 1932; Escuder, 1936; Rodrigues-Lopes, 1936).

Wilson in 1954 found a factor which in dilution agglutinated spermatozoa in blood and seminal plasma of 2 men out of 100 sterile men.

In 1954 Rumke found two men with sperm agglutinins in their blood, among 80 men with azoospermia or extreme oligozoospermia.

In 1956 Wilson reported that sperm agglutination factor in men's blood was absorbable by semen and active after heating at 56°C for 30 minutes. The man's spermatozoa quickly lost their motility in cervical mucus in vitro and at post coital test cervical secretion contained only a few spermatozoa.

all of which were immotile or showed sluggish motility. Two of the men's wives promptly conceived after artificial insemination with donor's semen.

On the basis of these observations he concluded that sperm agglutination due to auto antibodies could be a cause of sterility.

Cruickshank and Stuart Smith (1959) observed that infection could be one of the factors responsible for producing sperm agglutination.

In 1960 Behrman et al presented evidence that ABO blood group incompatibility may play a role in fertility.

Nakabayashi et al in 1961 examined sera from infertile men and from both fertile & infertile women and found sperm agglutinins in a small proportion of sera from individuals in all groups with highest incidence being in sera from infertile women.

Rao and Sadri in 1961 pointed out the existence of antigens specific to spermatozoa.

Franklin & Dukes in 1964 for the first time demonstrated the sperm agglutinating activity in serum of couple with unexplained infertility by using

micro-agglutination techniques. They had also carried out the fluorescent antibody studies and had demonstrated that agglutination was the result of an antigen antibody reaction and not merely a spontaneous aggregation.

They had studied 89 patients for the incidence of circulating antispermatozoal antibodies and found higher incidence i.e. 78.9% of anti-spermatozoal antibodies in women of unexplained infertility and 10.37 percent in case where there was organic reason responsible for infertility, in 11.8 percent of the patients of known fertility and in 4.27 percent of the patients of unknown fertility. It was concluded by them that the presence of circulating sperm agglutinating antibody in women is correlated with unexplained infertility. Statistically the result indicated a strong correlation between the existence of circulating antispermatozoal antibody and unexplained infertility.

Franklin and Dukes in 1964b again carried out a study in 214 patients, which they had grouped under 5 group i.e. an additional group of patients having secondary sterility. They found an incidence of 72.1 percent of sperm agglutinating antibodies in cases of unexplained infertility and 8.4 percent

incidence in cases of organic infertility, 5.7 percent incidence in fertile group and unknown fertility while 5.6 percent incidence in patients of secondary sterility. They concluded that the circulating sperm agglutinating antibody, an immunologic process was responsible for the state of infertility.

Schwimmer, Ustay and Behrman in 1967 had carried out the study in 292 women and 176 men using modified Franklin & Dukes method. They had included an additional group of prostitutes. They observed that iso-agglutinins occurred in 37.5 percent of 64 couples with primary unexplained infertility and in 50% of 32 couples with secondary unexplained infertility as compared to 20% organic infertility. 7.9 percent of men of unexplained primary infertility had auto-agglutination. They reported a very high incidence of iso-agglutinins i.e. 72.9 percent of 48 prostitutes and had correlated this finding with decreased fertility status in these women.

Franklin & Dukes in 1968 had studied 487 patients for sperm agglutinating antibodies and reported a high incidence of positive sperm agglutination test in unexplained infertility. i.e. 67.2 percent of 67 cases, 15.9 percent in organic cause for infertility and 9.1 percent in fertile cases, while 20 percent in secondary infertility.

Isojima (1969) reported that sperm agglutination test was positive in 26.5 percent of cases having unexplained infertility, 22.4 percent of cases having organic infertility.

Israelstam in 1969 studied 45 couples with no obvious cause for infertility lasting for 1/2 to 9 years. He used the same technique as used by Franklin & Dukes for sperm agglutination in the sera with the exception that no attempt was made to standardize the number of sperm either by concentration or dilution as centrifugation of semen, lead to clumping of the spermatozoa. They found sperm agglutination in 29 percent of 45 infertile women, but only 3 cases i.e. 7 percent, the sperm agglutination was due to serum factor.

In 1969 Srivannaboon et al reported 13.5 percent sperm agglutinating antibody in unexplained infertility in female using modified Franklin Dukes technique.

Glass and Vaidya in 1970 demonstrated that 24 out of 122, i.e. 20 percent women with unexplained infertility had positive sperm agglutination test. Nine of these women became pregnant after condom used by their husbands for 3-6 months.

Vaidya & Glass (1971) further studied 110 infertile women for sperm agglutinating and immobilizing antibodies. Six women out of 30 women with unexplained infertility had positive sperm agglutination test, while two of these women became pregnant during the follow-up period after using condom therapy. All 10 women with an organic cause responsible for infertility had both negative sperm agglutination and sperm immobilization tests.

Kolodny et al in 1971 studied 300 women for presence of circulating sperm agglutinating antibodies. Eleven of 78 infertile women (14.1%), 15 of 86 prostitutes (17.4%), 3 of 71 nuns (4.2%) and none of 65 normally fertile women were found to have sperm agglutinating activity in their sera.

Ansbacher (1971) reported 17.2 percent of 377 infertile couples possessed sperm antibodies of which 11.4 percent were demonstrated in wife and 5.8 percent in husband.

Isojima et al (1972) reported that sperm agglutinating antibodies were present in sera of 37.5 percent of women having unexplained infertility, 19.4 percent in organic infertility.

Mukerjee et al in 1973 studied the role antispermatozoal antibodies in cases of unexplained sterility of 3-22 years duration and was found positive result in 19 percent of patients having unexplained primary infertility and 10 percent of patients having organic cause for infertility. None of the patients with known fertility had such antibodies.

Shulman in 1978 reported the incidence of positive sperm agglutination in 15 percent of 389 infertile women and in 5 percent of the 397 infertile men, while none in the fertile couple.

Mukerjee et al in 1978 studied 150 cases of primary and secondary sterility and 10 fertile cases. They reported that sperm agglutination was positive in 10.1 percent cases of primary infertility with unexplained cause while 5 percent cases of organic infertility, none in control cases of known fertility.

Upadhyay et al in 1979 studied 900 couples having primary and secondary sterility and observed the incidence of sperm agglutination in 28.6 percent couples having primary infertility and 16 percent of couples having secondary infertility while none in

fertile couples. Out of 145 couples 20 cases became pregnant after having condom therapy and corticosteroid therapy.

Mishra & Dass in 1981 found positive sperm agglutination in 10 percent of cases of primary unexplained infertility.

Srivannaboon et al in 1982 reported the incidence of positive sperm agglutination in 19.5 percent of cases varying from weekly to strongly positive.

These 147 women with positive test were selected for therapy. Prevention of contact of semen with the female genital tract was done by three alternative viz. abstinence, withdrawal or condom therapy. The antibody test had fallen within 3 months to 18 months interval. Twenty five women out of 85 couple became pregnant after condom therapy. Misra et al (1984) reported the incidence of sperm agglutination in serum in 12% of cases with unexplained infertility.

Antibodies in Genital secretions against spermatozoa surface membrane antigen -

The concept of fertility inhibiting effect of sperm antibodies presupposes that the antibodies are

present in the genital tract during the fertilization process. The occurrence of sperm antibodies in genital secretions from women and their possible association with infertility has been investigated in rather a few studies principally performed on secretions of lower genital tract i.e. cervix and vagina.

Immune Potential of female genital tract -

During the last decade or so it has been realized that mucosal membrane e.g. gastro-intestinal tract and respiratory system, contain a local immune system, which in cellular, physico-chemical and functional terms constitutes a distinct entity, markedly independent of the systemic immune apparatus.

Tomasi (1965) and Ogra (1968) has suggested that local appearance of antibody may be due to a mechanism independent of circulatory antibody. They demonstrated that the uterine cervix is biologically similar to these glands in that it serves as a barrier preventing the bacteria of vagina from entering the sterile uterine cavity and also produce a local immune response. Although vagina itself has been suggested on being capable of local antibody production against spermatozoa (Parson, 1940 and Beurman, 1965).

Further potential significance of this immune mechanism of cervix in fertility reduction, by the production of local antibodies is supported by Tomasi and Biennenstock (1968), Dayton et al (1971), Tomasi and Grey (1972), Hanson and Brandtzaeg (1973), Saltaft (1973), Mestecky et al (1978).

Omran and Hulka (1971) also demonstrated the presence of necessary ingredients for local antibody production in the cervical mucus.

The major characteristics of these mucosal membranes and their secretions are -

1. The predominance of IgA producing plasma cells in the submucosa (unlike the systemic immune system in which plasma cell of IgG type constitute the majority of immunoglobulin producing cells).
2. An ability to synthesize IgA in vitro.
3. A ratio between IgG and IgA secretions about or below 1, reflecting the relative predominance of IgA (whereas in serum IgA makes up a minor fraction of immunoglobulin with IgG to IgA ratio 4).
4. A unique physico-chemical structure of IgA, polymeric, containing low molecular weight secretory component and having total molecular

weight 390,000 and sedimentation coefficient of 11 S for 80 to 90 percent of secretions.

(The IgA in human sera is principally monomeric, with sedimentation coefficient of 7 S).

5. The development of antibodies after local immunization may occurs earlier and in higher titer than in serum, may be even without detectable antibodies in serum.

#### Immune Potential of Cervix and Vagina -

The immune potential of the female genital tract is discussed according to the following criteria -

1. Occurrence and types of submucosal plasma cells.
2. Demonstration of immunoglobulin synthesis in vitro.
3. Quantification of immunoglobulin contents of the secretions, with special emphasis on the IgG/IgA ratio.
4. Presence of secretory IgA (11 S - IgA).
5. Occurrence of immunoglobulin class after experimental immunization or infection.

Local production of antibodies following infection or local deposition of antigen in the vagina or cervix has been demonstrated in several animal

studies indicating that the cervical and vaginal domain possess a secretory immune potential by Kerr (1955), Bell & Wolf (1967), Omaran & Hulka (1971), Wilkie et al (1972), Corbeil et al (1974b), Mc Amilty & Morton (1978), Yang & Schumacher (1979).

Tourville et al (1970), Lippes et al (1970), Hutcheson et al (1974), Rebello et al (1975), demonstrated IgG, IgA, IgM in cervical tissue interstitially as well as in submucosal plasma cells by several direct immuno-flourescent techniques. Immunoglobulin IgM has been demonstrated only occasionally and in very low concentration 0.01 - 0.035 mg/ml by Messon et al (1969), Waldman et al (1972a), Schumacher (1973a), Conghan and Skinner, (1977a).

Behrman and Lieberman in 1973 observed the synthesis of IgG and IgA in all supernatants of tissue culture of cervical biopsy from infertile women with an average IgG & IgM ratio of 2.3 (0.6 - 5.0) as assayed by rocket immuno-electrophoresis with an 11 S - IgA as standard.

Cervical mucus has been subjected to extensive immunological, physico-chemical and ultra-structural investigation.

Blandau and Moghissi (1973), Elstein et al (1973), Insler and Bettendorf (1977) demonstrated that cervical mucus is a mucinous gel consisting of macromolecular network of long glycoprotein bound together by cross linking. The intermicellar space filled with cervical plasma (a protein rich fluid).

Schumacher (1973 a, b) showed a characteristic cyclic variation in the concentration of IgG and IgA, being highest during the luteal phase and early follicular phase and reaching a typical minimal around mid-cycle. The mean concentration of IgG and IgA varied between 0.1 - 6.0 and 0.06 - 1.4 mg/ml respectively while in serum varies 8 - 16 and 1.4 - 4.0 mg/ml respectively.

The cyclical change in the concentration of IgG and IgA probably due to dilution with the varying amounts of cervical mucus secreted rather than to a hormonal influence on the rate of plasma cell synthesis of immunoglobulin.

The IgG and IgA ratio seems to change during the menstrual cycle, being approximately 6 in the luteal phase of early follicular phase but around 2 in mid cycle by Hulka and Omaron (1969); Schumacher (1973 a, b).

Hutcheson et al in 1974 observed an especially high occurrence of IgA producing plasma cells in cervical biopsy from women with unexplained infertility and concluded that immunological factors may be responsible for infertility in these women.

Plasma cells are observed principally in the endocervical transformation zone between the squamous and columnar epithelium, in which location a significant relative dominance of IgA producing plasma cells (80 percent) was demonstrated by Rubello et al (1975).

These results seems to indicate that the concentration of complement in cervical mucus is not high enough for elicitation of a significant sperm immobilizing activity. The biological significance of such low concentration of complement is uncertain.

#### Occurrence of sperm antibodies in cervical mucus -

Though the antigenicity of sperm and seminal fluid constituents has been known since a very long time, their implication in the etiology of unexplained infertility has acquired a considerable importance in recent years.

Franklin and Dukes (1964 a) provide the first detail information on sperm agglutinating activity in

female with unexplained infertility. He demonstrated that material from the reproductive tract contain antigens capable of inducing the formation of antibodies, detectable by the casual serological tests.

Parish et al in 1967 have demonstrated the antibodies cytotoxic to spermatozoa in 3 out of 11 women (27.27%). They observed that infertility because of sensitization to seminal cytotoxic IgG antispermatozoal antibodies in the cervical mucus.

Parish and Ward in 1968 demonstrated the antisperm antibodies in the cervical mucus in 3 out of 48 cases i.e. 6.225 percent. They also demonstrated the relationship of antisperm antibodies with blood group of women and specificity of antispermatozoal reactions in the cervical mucus and serum of infertile women. They found that there were two different cytotoxic antibodies and three harmless antibodies reacting with spermatozoa. One woman had an IgG cytotoxic antibody in her serum and cervical mucus reacting strongly with the head of spermatozoa and disrupted them in the presence of complement. The cervical mucus of second woman containing cytotoxic IgG antibodies reacting with antigen all over the spermatozoal surface, immobilizing them without causing morphological changes.

Three harmless antibodies were IgM and IgA globulins that were specific for antigen in the seminal plasma which coated the spermatozoa.

Eyqueen and D'Almeida in 1973 tested cervical mucus in 59 women with unexplained infertility. In seven women the sperm antibodies were demonstrated by the immunoflourescent technique and in twelve women by a spermatoxic test. No details of technique and titers were described in this report.

Coelingh et al in 1974 demonstrated the positive result in 3 out of 13 women with unexplained infertility, i.e. 23.77 percent in cervical mucus by immunoflourescent techniques. They reported the concept of local production of sperm antibodies.

In 1974 Shulman demonstrated sperm agglutinating activity in extracts of cervical mucus from infertile women.

Shulman in 1975 found the positive sperm agglutinating activity in the cervical mucus. They studied 1000 couple of unexplained infertility and found positive result in 15 percent of cases.

Even more so, in a prospective search that was initiated after the in vitro observation, sperm agglutination of the head to head form was observed

in the post coital cervical mucus in three tests. The percentage of sperm agglutination ranged from 3 to 10 percent. He demonstrated first time the sperm agglutination in the post coital test itself. However, these post coital sperm was all immotile. They found sperm agglutination in cervical mucus only not in sera in 3 of these women.

Their finding also suggested that sperm agglutinins is produced locally i.e. in the cervical tissue and eventual leakage of sperm agglutinin from the cervix to general circulation give rise to production of antibodies in sera.

Kremer and Jager in 1976 demonstrated the sperm agglutination (head to head) at microscope titer of 32 in the aqueous fraction of cervical mucus.

Sudo et al in 1977 in subsequent study of cervical mucus extract from 450 infertile women, he and his co-worker detected twelve positive secretion (2.7 percent) by the tube slide agglutination test. Nine of these twelve women had concurrent sperm agglutinating activity in serum, while in cervical mucus from 58 fertile women sperm agglutinating activity was not observed in any case.

Kremer and co-worker in 1977 have demonstrated 7 women with sperm antibodies in cervical mucus.

Ingerslev and Hjort (1979); Ingerslev and Ingerslev (1980) revealed that 5.8 percent of a consecutive series of women from infertile couples had sperm agglutinating antibodies in serum.

Ingerslev (1980) investigated cervical mucus from 21 infertile women with sperm agglutinating antibodies in serum, by applying the tray agglutination technique to bromeline treated cervical mucus. Eight women out of 21 (38 percent) had concurrent sperm agglutinating antibodies in ovulatory cervical mucus.

Moghissi et al (1980) studied 172 infertile women and detected significantly higher frequency of sperm agglutinating cervical secretion (16.3 percent). They also found a negative serum in 22 of the 28 women with sperm agglutinating activity in cervical mucus.

Sperm antibodies in serum probably appear as a consequence of sperm antigens being exposed to the immune system in the genital tract. It was therefore, reasonable to believe that antibodies should be present concomittantly in serum and genital secretions.

However, several report have demonstrated that only 40 to 60 percents of women with sperm agglutinating antibodies in serum have detectable antibodies in the cervical mucus (Shulman and Guerrero, 1978; Moghissi et al, 1980; Ingerslev, 1980).

Misra and Dass (1981) showed higher titer of antisperm antibodies with increasing duration of infertility.

Misra et al in 1984 studied 100 women having unexplained primary infertility and was found 12 out of 100 cases showed antisperm antibodies in serum by Franklin and Dukes test and out of these, 8 cases showed positive result in cervical mucus by micro double diffusion agar precipitation test. None of the control group showed positive result.

They also found that out of 8 women, 5 had positive test (62.5 percent) both in serum and cervical mucus and 3 had positive test in cervical mucus only.

Mukerjee et al in 1984 demonstrated the presence of antibodies in cervical mucus in 64 percent cases of unexplained primary infertility by microscopic agglutination test while cases of control group it was positive in 41.1 percent.

They also observed the antisperm antibodies in cervical mucus by macroscopic agglutination test and was found 60 percent cases of unexplained primary infertility showed positive results, while control group showed positive result in 58.8 percent of cases.

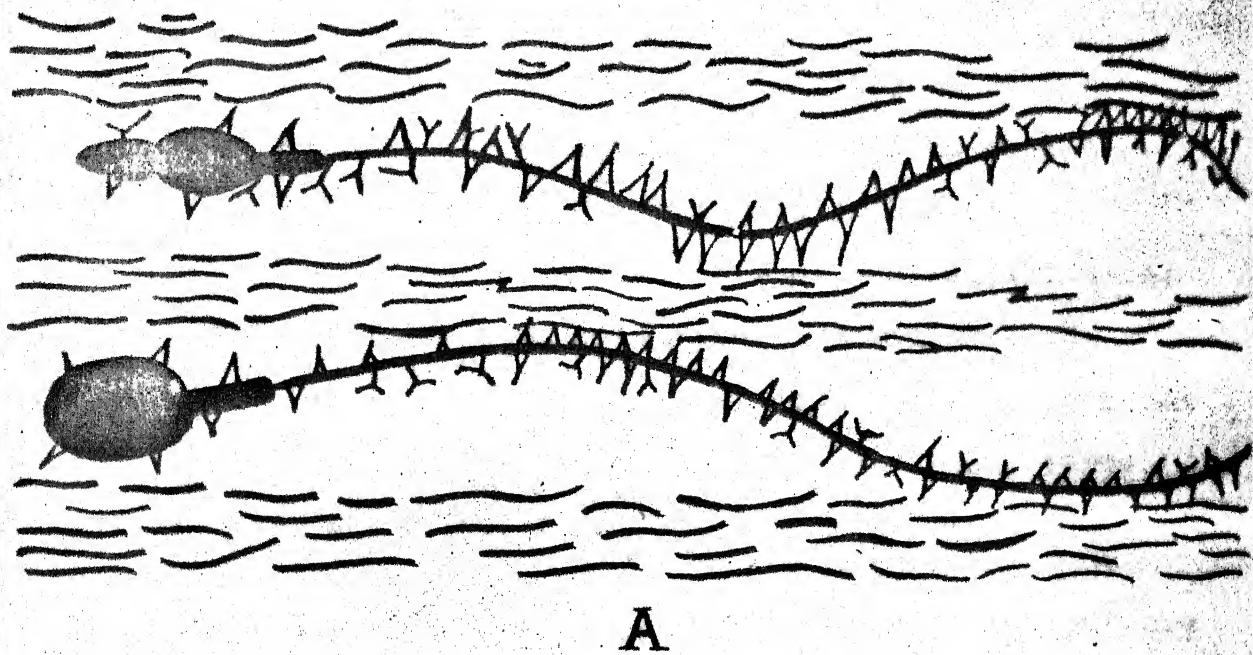
They also observed the relationship of development of antisperm antibodies in cervical mucus and serum with age of the women, duration of infertility and result of post coital test and they found the high incidence of antisperm antibodies in cervical mucus in women with longer duration of infertility.

#### Basis of sperm agglutination -

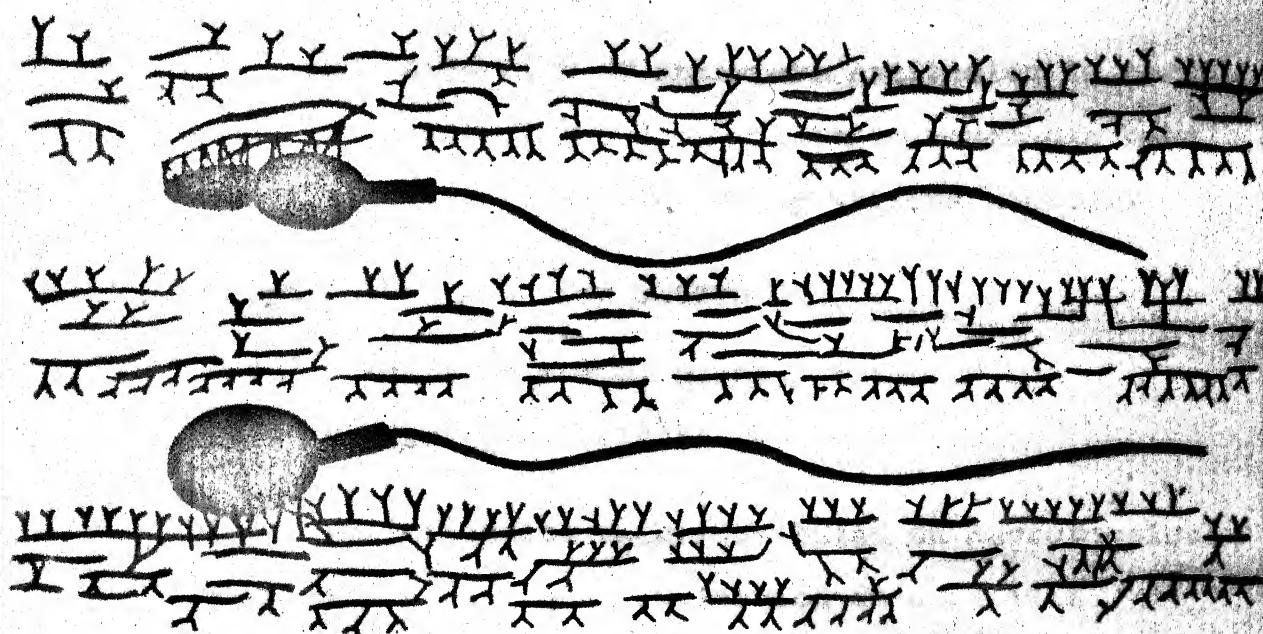
It is the agglutination of spermatozoa by the antispermatozoal antibodies. The antigens are present on the surface membrane of the human spermatozoa. The antibodies are present in the serum or in cervical mucus of infertile women (Fig. 2).

#### Pattern of sperm agglutination -

Microscopically examination of sperm agglutinates was used to discriminate between antibodies of apparently different specificity.



A



B

**FIGURE NO.2. HYPOTHETIC ATTACHMENT OF SPERMATOZOA TO THE GLYCOPROTEIN MICELLES BY MEANS OF ANTISPERM ANTIBODIES.**  
**A. ANTIBODIES ON SPERMATOZOA.**  
**B. ANTIBODIES IN CERVICAL MUCUS.**

The spermatozoa thus agglutinated either head-to-head, tail-to-tail or tail-tip to tail-tip. In addition, a mode of agglutination had been described in which head as well as tail agglutinates were seen (mixed agglutination) and another type where heads and tail both seem to be bound each other (tangled agglutination) (Rumke and Hellingsa, 1959; Rose et al, 1976), (Fig. 3).

Technique for detection of antibodies against surface antigens of spermatozoa in serum and cervical mucus -

The detection of antisperm antibodies in serum and in the cervical mucus of infertile women has been investigated extensively by using various methods.

1. Sperm agglutination method

(a) Kibrick or K.B.M. method, detected by Kibrick et al (1952).

Gelatin agglutination technique based on macroscopical observation of sperm agglutination in a gelatin medium also called macroscopic agglutination test of cervical mucus.

(b) Franklin Dukes method (Tube slide agglutination technique)

OR

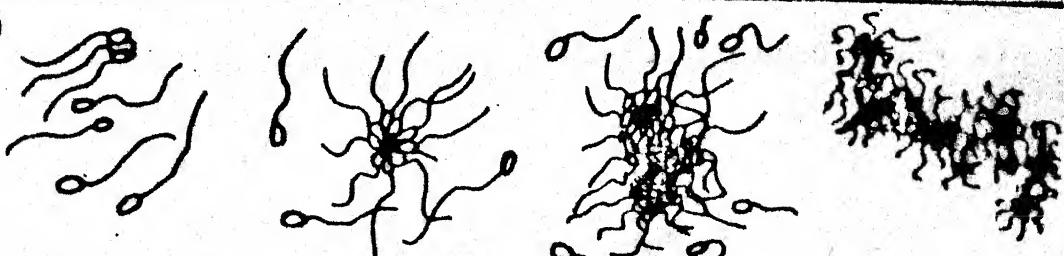
Micro scale test of cervical mucus extract.

FIG. NO.-2

DEGREE OF AGGLUTINATION

PARTS INVOLVED	1. ISOLATED ( $<10$ SPERM/ AGGLUTINATE, MANY FREE SPERM)	2. MODERATE ( $10-50$ SPERM/ AGGLUTINATE, FREE SPERM)	3. LARGE ( $>50$ SPERM, AGGLUTINATED, SOME SPERM & AGGLUTINATES STILL FREE)	4. GROSS (ALL SPERM AGGLUTINATED, INTERCONNECTED)
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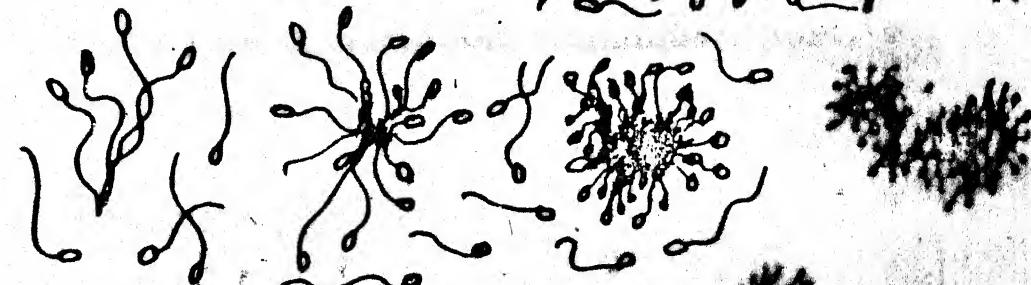
A. HEAD-TO-HEAD



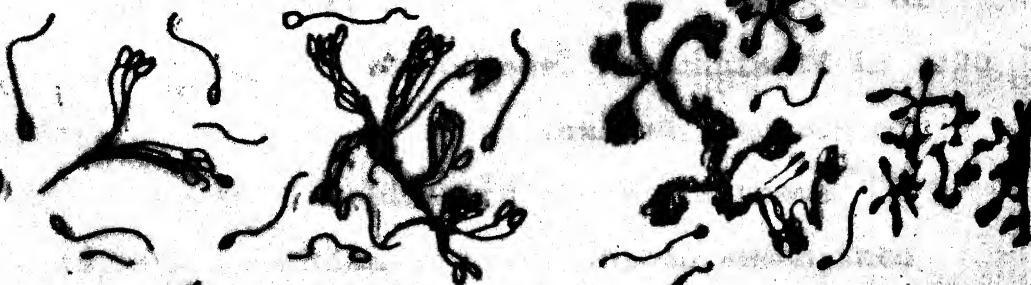
B. TAIL-TO-TAIL  
HEADS ARE  
SEEN TO BE FREE  
& MOVE CLEAR OF  
AGGLUTINATES



C. TAIL-TIP-TO-TAIL  
-TIP



D. MIXED (CLEAR  
HEAD-TO-HEAD  
& TAIL-TO-TAIL  
AGGLUTINATIONS),



E. TANGLE (HEADS  
-TAILS ENmeshed,  
HEADS ARE  
NOT CLEAR OF  
AGGLUTINATES AS  
THEY ARE IN  
TAIL-TO-TAIL  
AGGLUTINATIONS)

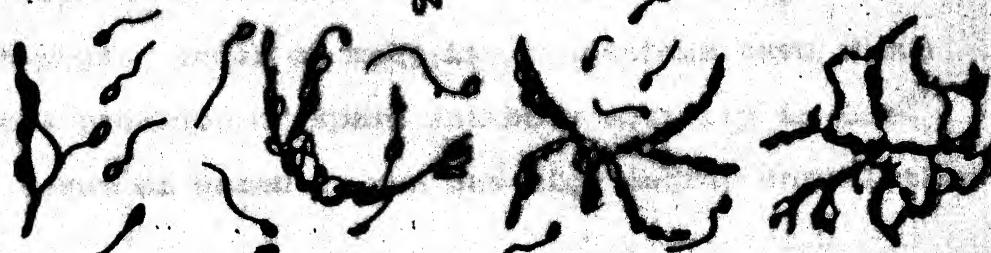


DIAGRAM SHOWING VARIOUS PATTERN  
OF AGGLUTINATION

40

(originally described by Franklin Dukes, 1964 a, b). A micro-agglutination method in which a drop is removed from a test mixture of semen and patient serum or semen and cervical mucus of women and subsequently examined under cover slip.

(c) Tray agglutination technique (Friberg, 1974 a).

2. Immobilization method (Isojima et al, 1968).

based on quantitative evaluation of sperm immobilizing effect of a test serum after the addition of guinea-pig complement.

3. Cytotoxicity technique (Hammerlynk & Rumke, 1968).

4. Indirect Immunofluorescent technique (Hjort, T., Hensen, K.B., 1971).

Clinical significance -

The investigation of immunologic methods of controlling fertility is not new. Metchnikoff in 1900 demonstrated the production of antibody capable of agglutinating and immobilizing the spermatozoa following the injection of heterologous semen into guinea-pigs. Later on many investigations have shown that the presence of sperm antibody activity in the blood serum of women or men has high degree correlation

with a concomitant problem of infertility by Baskin (1932); Escuder (1936); Rodroques-Lopes (1936); Franklin Dukes (1964 a, b); F-D, (1968).

Since the presence of sperm antibodies in serum sample was an established fact, Shulman in 1975 first of all searched for such activity in the cervical mucus of women and in the seminal plasma of men.

Later on it has been proved by various workers that sperm antibodies in serum probably appears as a consequence of sperm antigen being exposed to the immune system in genital tract, and they found the higher incidence of sperm antibodies in cervical mucus of unexplained infertile women while the serum of these women may be negative for such antibodies. Shulman (1974); Shulman (1975); Sudo Shulman (1977); Ingerslev & Hjort (1979); Ingerslev (1980); Moghissai (1980); Misra et al (1984); Mukerjee et al (1984).

It has been amply demonstrated that materials from the reproductive tract contain antigens capable of inducing formation of antibodies detectable by usual serological test.

The majority of these reports have involved the antifertility aspects or contraceptive potential of these immunologic phenomena.

**MATERIAL AND METHODS**

### MATERIAL AND METHODS

The present study was carried out in the Department of Obstetrics and Gynaecology and the Department of Pathology, M.L.B. Medical College and Hospital, Jhansi, over a period of one year starting from July 1984 to June 1985.

The study comprised of the women attending the out-door clinic of the Department of Obstetrics and Gynaecology, M.L.B. Medical College Hospital, Jhansi, for infertility - primary or secondary.

Selection of cases - Ninety six women from couples being treated or having been treated for infertility were selected. All the women utilized in this study group were assigned to one of the following categories strictly on the basis of history and clinical evaluation.

Group I - women of known fertility.

This group included women who delivered at least one alive child within 3 year of the time of inclusion in this study.

Group II - Included infertile women with no demonstrable organic cause for infertility except poor post-coital test.

Minimal criteria include 2 years without contraception, with normal pelvic finding, normal endometrial biopsy, normal Rubin's test, hysterosalpingogram, normal husband's seminogram and essential normal pertinent blood studies on the patients.

This group is further divided into

A. Primary infertility,

B. Secondary infertility.

History - Detail history from each partner was taken separately, i.e. age, religion, occupation, socio-economic status, smoking or drinking habits along with their present history related to infertility, e.g. menstrual disorder, discharge per vagina, backache, dyspareunia, urinary trouble.

Marital history - Duration of marriage, period of stay with husband, contraceptive practice if any, frequency and timing of coitus in relation to menstrual cycle, dyspareunia, premature ejaculation,

impotence were the partners separated from each other for significant period of time, detail about all these things were asked from each partner.

**Menstrual history** - The women were asked detail about the menstrual cycle, duration of flow, amount of blood loss, passage of clots, pain and last menstrual period.

**Obstetrical history** - Women those having secondary infertility were asked detail regarding previous pregnancy, abortion, delivery, periparturient fever, post abortal fever and any complication during or after delivery or abortion.

**Past history** - Every women were asked about the medical or surgical history in the past. Medical history - including history of mumps gonorrhoea, syphilis, tuberculosis, allergy, diabetes or pelvic infection, eczema. Surgical history - includes history of any operation on or near the genital tract.

**Family history** - Detail family history were taken from each partner pointing towards infertility, tuberculosis, diabetes mellitus, syphilis.

**Examination** -

All the women were further evaluated clinically by general, systemic and local gynaecological

### 1. General Examination -

A thorough general examination was done of all the cases with special emphasis on detection of obesity, pallor, abnormal breast development and hirsutism.

### 2. Systemic Examination - was done to exclude any underlying lung and heart pathology.

### 3. Local Examination - was done to note any sign of hypoplasia or malformation.

(a) Per speculum examination - was done to see the condition of cervix, vagina and any discharge per vaginum.

(b) Per vaginal examination -

Bimanual examination was done to note the local condition of uterus and adenex and any associated pathology.

### Investigation -

Routine examination of blood for haemoglobin, total and differential leucocyte, erythrocyte sedimentation rate and urine examination for albumin and sugar was done in all women.

Special investigation -

Blood sugar, VDRL, ABO, Rh grouping, X-ray chest was done in all the women of study group. Vaginal swab for culture and sensitivity was done in women complaining of discharge per vagina, or in which it is significant.

Every women with infertility had pre-menstrual endometrial biopsy, post menstrual tubal testing. In case of demonstrable pathology elaborate tests were done to ascertain the cause of infertility like hysterosalpingography and diagnostic leproscopy.

Post coital test was done in as many women as possible. In male, semen analysis was done in every case.

METHOD

For detection of sperm agglutinating antibodies in cervical mucus and serum microscale test of cervical mucus extract or tube slide agglutination technique described by Franklin Dukes, (1964 a, b) was used in slightly modified way.

Sample collection -

1. Cervical mucus aspiration -

The women were instructed to maintain the complete sexual abstinence for at least two days prior to cervical mucus aspiration. Clear endocervical mucus of pre-ovulatory phase on the 12th or 13th or 14th day of menstrual cycle was aspirated by the glass vaginal pippet.

Before aspiration of cervical mucus, external os was clean with dry cotton to avoid the contamination with vaginal fluid. Maximum amount of cervical mucus that could be possible was obtained without drawing any evident blood. The aspirated cervical mucus was transferred to autoclave glass test tube.

Cervical mucus pH values - pH of the aspirated cervical mucus was tested by pH indicator paper before and after extraction of cervical mucus.

Cervical mucus extraction - Double amount of sorenson's buffer (pH - 7.2) were added to the cervical mucus and agitated frequently for two hours at room temperature. Samples were then centrifuged at 6,000 rpm for 30 minutes with

centrifuge. The supernatant (cervical mucus extract) were then transferred to the other test tube.

Serum collection -

For this all women underwent venipuncture and 5 ml. of venous blood was taken and allowed to clot. Serum was separated by centrifugation at 6,000 rpm for 10 minutes. The serum was then incubated at 56°C for 1/2 an hour to destroy the complement in hot water bath. The serum of two different strength was used like undiluted N and diluted N/10 strength. The serum had been diluted to avoid the non-specific reaction with undiluted serum (Shulman, 1975), and to obtain a titer for sperm antibody. The dilution of serum was done with normal saline. Diluted and undiluted serum was placed in different serological tube and were labelled accordingly. The normal saline was also taken in another test tube as a control to screen out any non-specific reactions.

Semen collection -

Semen sample with more than  $60 \times 10^6$  cells per millilitre, with over 60 percent motility were chosen.

Husband's semen sample was obtained by masturbation in a sterile petri dish after 2 days of abstinence, within an hour of initiation of testing procedure. Upon receipt in the laboratory of the ejaculated specimen, complete semen analysis was performed by a standard method which include volume, colour, viscosity, pH, liquification time, percentage of motile sperm, count per milli-litre and morphology immature form, abnormal sperm, presence of leucocyte, bacteria and pus cells. Semen sample were adjusted by dilution or concentration to a total number of approximately 50 million spermatozoa per milli-litre.

Procedure -

Microscale test of cervical mucus extracts -

A volume of 0.01 ml of  $60 \times 10^6$  cells per milli-litre adjusted semen sample was pipetted from the bottom of the serological tube, over this small semen sample 0.1 ml of cervical mucus extract was dropped. After gentle shaking for few seconds, the tubes were incubated at  $37^{\circ}\text{C}$  for 2 hours. Immediately, after 1 hr and 2 hr, one drop of cervical mucus sperm mixture was pipetted on to a slide, covered by cover slip and observed under the microscope in high power field for agglutination.

For a control test mixture 0.1 ml of normal saline was dropped over .01 ml of semen and incubate at the same temperature for same period. One drop of this mixture was taken after 1 hr and 2 hr, droped on the slide and observed under microscope under high power field to exclude the other cause of agglutination.

Interpretation -

Agglutinated sperm, non-agglutinated motile and immotile sperm were counted. Twelve high power field were examined.

Total of about 100 motile sperm were counted. The ratio of motile agglutinated sperm to total motile sperm was calculated. More than 10 percent agglutination was considered as a positive microscale agglutination.

2. Tube slide agglutination technique - or

Franklin Dukes Test of serum agglutination -

Test was carried out in 3 serological tube as follows :

Test tube I - 0.5 ml of woman serum +  
0.1 ml of semen.

Tube II - 0.5 ml of women serum of  $\frac{N}{10}$  dilution  
+  
0.1 ml of semen.

Tube III - 0.5 ml of normal saline  
+  
0.1 ml of semen.

All the test tubes were incubated at  $37^{\circ}\text{C}$  for 2 hrs. A drop is drawn from each test tube immediately after 1 hr and after 2 hr, on the slide, covered by cover slip and examined under microscope.

Interpretation -

Slide was examined for agglutinated sperm, motile and immotile sperm. Evidence of positive agglutination was considered if 2 or more than two spermatozoa aggregated per high power field.

Strength of agglutination was graded as follows -

1+ 2 or 3 sperm agglutinated several high power field.

2+ 2 or 3 sperm agglutinated per high power field.

3+ Large aggregate per high power field.

Aggregate to dead sperm or living sperm to debris was considered as non-specific. An interwoven mesh work of sluggish sperm was considered as a non-specific reaction.

Pattern of agglutination - The pattern of agglutination of sperm was recorded.

1. Head to head,
2. Tail to tail,
3. Mixed agglutination - Head as well as tail.

Treatment -

Theoretical possibilities for treating infertility due to sperm antibodies in female of an infertile couple include -

1. Occlusion therapy, in which the use of a condom prevents exposure of the female to sperm antigens.
2. Treatment with corticosteroid, supressing the immune response.
3. Estrogen medication, which may reduce the antibody titer in cervical mucus and improve the penetrating ability of spermatozoa in women with sperm-agglutinating antibodies in cervical mucus (Ingerslev, 1980).

4. Eradication of genital infections, on the assumption that such affections may be of importance for initiating and maintaining immune reactions to spermatozoa.

The women in which sperm agglutination was demonstrated in cervical mucus or in serum, or in both, condome therapy along with corticosteroid is advised for 6 wks to 3 months.

Tab. Prednisolone is given 5 mg thrice daily for 15 days then 5 mg tab. twice daily for next 15 days then once daily for another 15 days. Patients were asked for follow-up every week if they had any side effect with corticosteroid therapy and general check-up after 7 days.

The women having sperm agglutinating antibodies in only cervical mucus were kept on estrogen therapy. Tab. ethinyl estradiol 50 µg daily given from 5th to 25th day of menstruation for 3 cycle.

After cessation of therapy for 3 months, women were asked to resume normal coitus.

Equipments required -

1. Glasswares -

- Glass syringes with I/V needles,
- Test tubes,
- Petri dish,
- Beaker,
- Slide,
- Cover slip,
- W.B.C. Pippet,
- Dropper,
- Neubauer's chamber,
- Glass pippet - 1 ml,
  - 0.1 ml,
  - 5 ml.

2. Test tube rack,

3. Centrifuging machine,

4. Hot water bath at 56°C,

5. Microscope,

6. Nitrazine pH paper,

7. Reagents - Semen diluting fluid,

- Normal saline,
- Sorensen's buffer (pH - 7.2).

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O B S E R V A T I O N S

O B S E R V A T I O N S

A total number of 106 women were studied for the occurrence of sperm agglutinating antibodies in the cervical mucus and serum of infertile women in one year period. These women were divided into two different groups as follows.

Table I  
Distribution of cases.

Group	Total No.of cases	%
Group I	10	9.44
Group II	96	90.56
Total	106	100.00

Group I included control cases of known fertility. All the 10 cases were having at least one alive child born within 3 years of study period. Test was done in non-pregnant state.

Group II included 96 women in which no demonstrable organic cause for infertility was present except poor post-coital test.

Table IIDistribution of cases according to type of infertility.

Type of infertility	Total No. of cases	%
1. Primary infertility	86	89.58
2. Secondary infertility	10	10.42
Total	96	100.00

Total number of 96 women were studied out of which 86 (89.58%) were having primary infertility, while only 10 (10.42%) were having secondary infertility.

Table IIIDistribution of women according to age.

Age group in years	Group II		Group I	
	Total No. of cases	%	Total No. of cases	%
18 - 20	2	2.08	1	10.00
21 - 25	30	31.20	2	20.00
26 - 30	45	46.87	3	30.00
31 - 35	18	18.75	4	40.00
36 - 40	1	1.04	-	-
Total	96	100.00	10	100.00

The patient's age varied from 18 to 40 years. The maximum number of cases of primary infertility were found between 26-30 yrs (46.87%). 78.13 percent of cases were between 21-30 yrs.

Maximum number of cases of control group were between 31-35 years.

Table IVDuration of infertility in study group.

Duration of infertility in years	Total No. of cases	%
2 - 5	24	25.00
6 - 10	50	52.08
11 - 15	20	20.84
16 - 20	2	2.08
<b>Total</b>	<b>96</b>	<b>100.00</b>

The duration of infertility ranged between 2 - 20 years, while maximum number of cases 44 (52.08%) falling in 6-10 years duration group.

**Table V**

Distribution of women according to duration of infertility in various types of infertility.

Duration of infertility in years	Primary infertility			Secondary infertility		
	Total No. of cases	%		Total No. of cases	%	
2 - 5	22	25.58		2	20.00	
6 - 10	44	51.16		6	60.00	
11 - 15	18	20.93		2	20.00	
16 - 20	2	2.33		-	-	
<b>Total</b>	<b>86</b>	<b>100.00</b>		<b>10</b>	<b>100.00</b>	

Maximum number of cases of primary and secondary infertility was between 6-10 years duration.

Table VI

Incidence of positive sperm agglutination test  
in serum of fertile and infertile women.

Group	Total No. of cases tested	No. of cases showing positive result	%
1. Control group (Group I)	10	Nil	-
2. Study group (Group II)	96	11	11.46
<b>Total</b>	<b>106</b>	<b>11</b>	

A total of 106 women were studied.

Eleven cases out of 96 having unexplained infertility had positive sperm agglutination test in serum, while none of the control cases showed positive result.

Table VIIIncidence of positive sperm agglutination test in sera of primary and secondary infertile women.

	Total No. of cases tested	Cases showing positive result	%
1. Primary infertility	86	10	11.62
2. Secondary infertility	10	1	10.00
Total	96	11	

Ten cases (11.62%) out of 86 having primary infertility showed positive result in sera while only 1 (10.00%) showed positive result in secondary infertility.

Table VIII

Relationship of sperm agglutination in sera with duration of infertility.

S.No.	Duration of infertility in years	Total No. of cases tested	Cases showing positive result	%
1.	2 - 5	24	1	4.16
2.	6 - 10	50	6	12.00
3.	11 - 15	20	4	20.00
4.	16 - 20	2	-	-

Table showing the percentage incidence of positive sperm agglutination test in sera. Maximum percentage of incidence was found between the duration of 11 - 15 yrs (20.00%).

Table IX

Sperm agglutinating activity in the undiluted N  
and diluted N/10 sera.

No. of cases	Immediately		After 1 hr		After 2 hr.	
	N	N/10	N	N/10	N	N/10
1.	-	-	+	+	+	+
2.	-	-	+	-	+	+
3.	+	+	+	+	+++	++
4.	-	-	+	+	++	+
5.	-	-	+	-	+	+
6.	-	-	+	-	++	+
7.	+	-	+	+	+++	++
8.	-	-	+	-	++	+
9.	-	-	+	+	++	+
10.	-	-	+	-	+	+
11.	-	-	+	+	+	+

In undiluted N sera of 11 infertile women with positive sperm agglutination test, sperm agglutinating activity was present immediately in 2 cases only, after 1 hr all the 11 cases showed

positive agglutinating activity, while at the end of 2 hr, 6 sera showed enhanced activity, in remaining 5 sera activity was stationary.

In N/10 diluted serum, 2 showed the positive sperm agglutinating activity immediately, 6 after 1 hr, and all the 11 after 2 hr.

Table X

Various patterns of sperm agglutination in sera.

Group	Case showed positive result	H-H	T-T	Mixed
II	11	6	2	3
%	100.00	54.54	18.18	27.27

Out of 11 cases showing positive sperm agglutination test in sera, 6 (54.54%) showed head-to-head agglutination, 2 (18.18%) tail-to-tail while 3 (27.27%) showed mixed pattern of agglutination.

Table XI

Incidence of positive sperm agglutination test in cervical mucus of fertile and infertile women.

Group	Total No. of cases studied	No. of cases showing positive result	%
1. Control group	10	Nil	-
2. Study group	96	26	27.29
Total	106	26	

No sperm agglutination was seen in control group, while 26 cases (27.29%) showed the sperm agglutination in cervical mucus of study group.

Table XII

Incidence of positive sperm agglutination test in cervical mucus in various types of infertility.

Type of infertility	Total No. of cases tested	%	Positive	%
1. Primary infertility	86	89.58	24	27.90
2. Secondary infertility	10	11.63	2	20.00
<b>Total</b>	<b>96</b>		<b>26</b>	

Twenty four out of 86 cases of primary infertility (27.90%) and 2 out of 10 cases of secondary infertility (20.00%) showed the positive sperm agglutination in cervical mucus.

Table XIII

Sperm agglutination in relation to duration of infertility in cervical mucus.

Duration of infertility in years	Total No. of cases tested	Cases showing positive results	%
2 - 5	24	3	12.50
6 - 10	50	16	32.00
11 - 15	20	6	30.00
16 - 20	2	1	50.00
<b>Total</b>	<b>96</b>	<b>26</b>	

Among the total number of cases studied according to duration of infertility, percentage incidence of positive result is shown in this table. Maximum number of positive result was seen between the duration of 6-10 yrs of infertility. Moreover, increasing duration of infertility was associated with higher percentage of positive cases.

Table XIV

Various patterns of sperm agglutination in cervical mucus.

Group	Total No.of cases showed positive result	H-H	T-T	Mixed
II	26	16	4	6
%	100.00	61.54	15.40	23.70

Out of 26, having positive sperm agglutinating activity in cervical mucus, 16 cases (61.54%) showed head-to-head pattern, 4 (15.40%) tail-to-tail, while 6 (23.70%) showed mixed pattern of agglutination in cervical mucus.

Table XV

Incidence of sperm agglutination in cervical mucus  
in women having positive agglutination in sera.

Total No. of cases with positive test in sera	No. of cases showing positive result in cx. mucus with positive sera	%
11	9	81.80

Out of 11 women having positive  
agglutination test in serum, 9 women showed  
positive agglutination in cervical mucus also  
(81.80%).

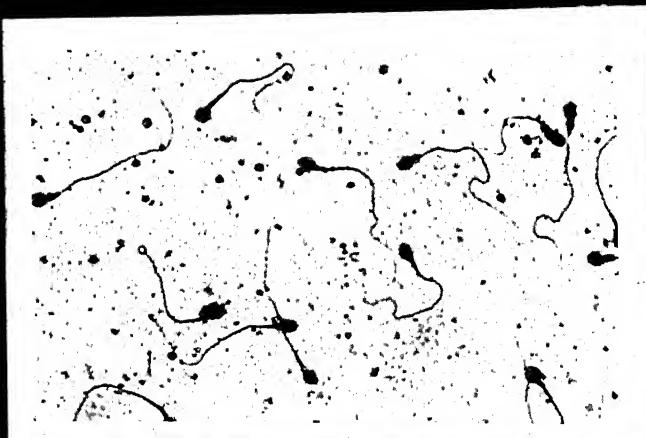
Table XVI

Incidence of sperm agglutination in sera with positive sperm agglutination in cervical mucus.

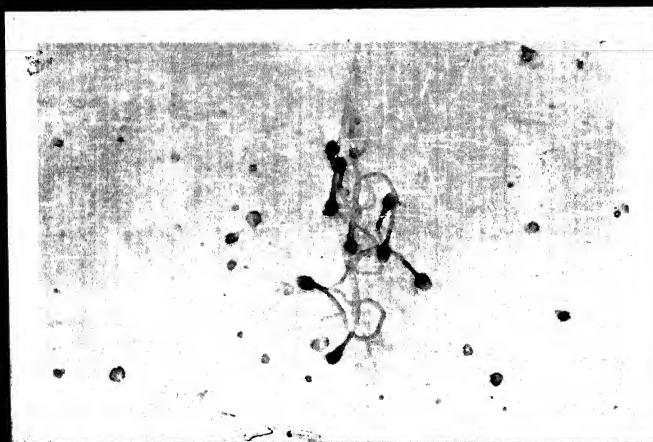
Total No. of cases with positive results in cervical mucus	No. of cases showing positive results in sera with positive cervical mucus	%
26	9	34.61

Out of 26 women having positive sperm agglutinating activity in cervical mucus, 9 women showed positive sperm agglutination in sera also (34.61%). Remaining 17 women showed sperm agglutinating activity in cervical mucus only.

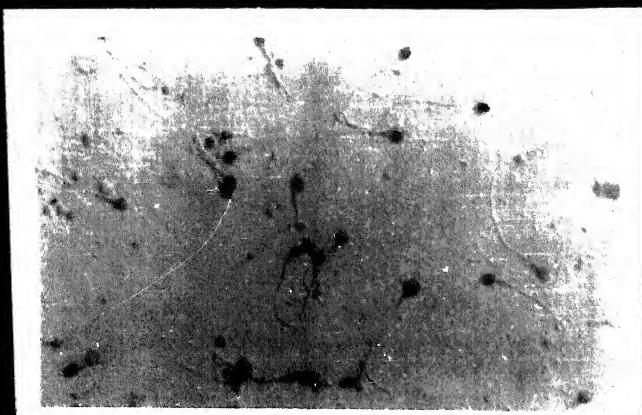
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Photograph showing negative  
result in cervical mucus  
1x50

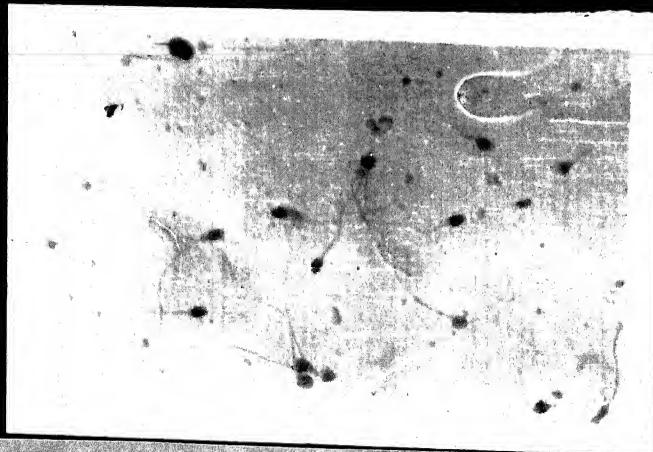


Photograph showing mixed pattern  
of sperm agglutination in  
cervical mucus  
1x50



Photograph showing head to head  
sperm agglutination in cervical  
mucus

1x50



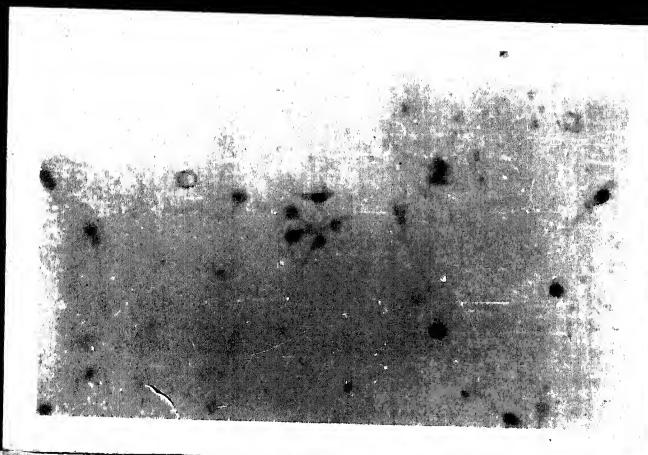
Photograph showing tail to tail  
agglutination in cervical mucus

1x50



Photograph showing negative  
result in serum

1x50



Photograph showing tail to tail  
agglutination in serum

DISCUSSION

## DISCUSSION

Despite all the emphasis on population control, infertility is certainly an important subject in its own rights. The infertile couple is viewed with ambivalence by the activist, who is concerned in utilizing every possible mechanism to reduce the population growth. However, humanistic view of protection of individual human rights dictates that barren couple are not to be denied the right to achieve their goal of parenthood.

Fertility has been one of the man's most desired attributes since the beginning of the recorded history. Fertility remains as a driving need for young couple today. In the past even on complete treatment of existing pathology, favourable results kept waiting and unexplained infertility in married couple still remains the crux of the problem of infertility.

When the couple had tried for at least one year to produce a pregnancy, and has been sure to include coitus at the time of each ovulation, they are defined by most workers as being infertile.

Immunological aspect of infertility helped the gynaecologist to know the exact etiology of unexplained infertility and also to provide the comprehensive treatment with better results.

Case where no cause can be attributed to the infertility, it is supposed that there is some auto-immune reaction between husband and wife which is responsible for infertility.

In considering the diverse mechanism that may operate in problems of spontaneous infertility, we can segregate a group of patients who are said to have "immune infertility". This term has been proposed to suggest that their fertility has been greatly reduced by the occurrence of antibody activity to spermatozoa.

We may expect that this concept surely become broadened in the future, to include antibodies to human ova. This will be based in part on the existing findings of Shiverse and colleagues with regard to the occurrence of acute antibodies to the zona pellucida and the preliminary findings that some women may well have such antibodies to their own zona (Shiverse and Dunbar, 1977).

Ten to thirty percent infertility is estimated to be explained on any organic or apparent basis (Shulman, 1972). Investigation indicate that immunological incompatability may be a causative factor in about 20% of couple whose infertility is otherwise unexplained.

There are two main cause of infertility as such

1. Presence of antibodies against spermatozoa in women.
2. Auto-immunity in man.

In the female antibodies are present in the genital tract due to local immune reaction against spermatozoa, or may be present in the serum because of systemic immune-reactions.

The sperm immobilizing phenomenon seen in the cervical mucus have been known since Sim's era. However, the microscopic agglutination of sperm in the cervical mucus has never been described or demonstrated before 1974, when Shulman demonstrated sperm agglutinating activity in extracts of cervical mucus from infertile women.

A recent report of Coeling (1974), Bennink and Menge (1974), stated that 'astonishingly' few

studies have been published on the detection of sperm antibodies in the secretion of genital tract fluids and obtained positive result of sperm agglutination in cervical mucus extract by microscopic agglutination test.

Keeping in view all these researches and interesting results, present study was undertaken. Only 'sperm agglutinating antibody' study has been done in case of unexplained infertility because it is much better established phenomenon and yet still creates controversy and confusion in some quarters.

For the purpose of discussion, a total of 106 women were studied. They were divided into two main groups.

Group I - included total number of 10 women of known fertility.

Group II - included 96 women having unexplained infertility (Table I).

Group II or study group has been further divided into two sub-groups depending on type of infertility.

A. Primary infertility - included 86 women.

B. Secondary infertility - included 10 women (Table II).

The cases were thoroughly investigated to exclude any organic cause for infertility.

Age of women varied from 18 - 40 yrs. Majority of cases were present between the age group 21 - 30 yrs (Table III). The duration of infertility ranged between 2 - 20 yrs, most of the cases had duration of infertility between 6 - 10 yrs (Table IV).

Sperm agglutination test was done in all 106 women in serum by Franklin Dukes method and in cervical mucus by microscale test of cervical mucus extract (tube slide agglutination technique). Result observed in different group was as follows.

A. Control group -

(i) Sperm agglutinating activity in serum -

In the present series none of the 10 women of control group showed sperm agglutinating activity in serum (Table VI).

Franklin & Dukes (1964 a) reported the incidence of 11.8% sperm agglutinating activity in sera of control group. Isojima et al (1972) observed the incidence of 45.8%, Jones et al (1973 a, 1974) 18%.

Shulman et al (1975) 2.6%, Ingerslev (1980) reported the incidence of 1.7% in their control group.

Table XVII

Incidence of sperm agglutinating activity in serum of control group by various workers.

Name of worker	Fertile group %
1. Franklin and Dukes (1964 a)	11.6
2. Isojima et al (1972)	45.8
3. Jones et al (1973)	18.0
4. Shulman et al (1975)	2.6
5. Petrusma et al (1976)	10.4
6. Ingerslev (1980)	1.7

Most of the Indian workers reported absence of antibodies in their control group (Hingorani et al, 1978; Mukerjee et al, 1978; Nanda and Pnigrahi, 1978; Upadhyay et al, 1979; Misra et al, 1984), while Joshi et al (1978) and

Misra et al (1981) reported an incidence of 3.3% and 2.8% respectively.

(ii) Sperm agglutinating activity in cervical mucus -

In the present study, none of the 10 women of known fertility showed the sperm agglutinating activity in cervical mucus (Table XI).

None of the positive result was obtained in cervical mucus by Shulman et al (1975), Sudo et al (1977), Ingerslev (1980), Moghissi et al (1980), Misra et al (1984) in their control group. Only one Indian worker, Mukerjee et al (1984) reported the incidence of 41% in their control group.

B. Study group (unexplained infertility)

(i) Sperm agglutinating activity in serum of study group -

In the present series sperm agglutinating activity in sera was present in 11.46% of cases having unexplained infertility (Table VI).

Table XVIII

Incidence of sperm agglutinating activity in sera  
of women having unexplained infertility.

Name of worker	Unexplained infertility %
1. Franklin and Dukes (1964 a)	78.9
2. Glass and Vaidya (1970)	19.7
3. Vaidya and Glass (1971)	20.0
4. Isojima et al (1972)	37.5
5. Jones et al (1973)	26.8
6. Mukerjee et al(1973)	19.0
7. Shulman et al (1975)	16.4
8. Petrunia et al(1976)	17.6
9. Mettler (1977)	16.8
10. Hingerani et al (1978)	20.0
11. Mukerjee et al(1978)	10.1
12. Nanda and Panigrahi (1978)	16.6
13. Ingerslev (1980)	6.3
14. Misra et al (1984)	12.0

Our values in unexplained group were almost consistent with Shulman et al (1975) 16.4%, Petrunia et al (1976 19.6%, Mettler et al (1977) 16.8%,

Mukerjee et al (1978) 10.1%, Nanda et al (1978)

16.6%, Misra et al (1984) 12.0%.

A positive reaction of sperm agglutination has been carefully defined in several reports by Boettcher and Hay (1969), Kolodny et al (1971), Shulman et al (1975) and Mettler (1977), but they had used different criteria. Thus, Boettcher et al (1971) counted the number of agglutinates, per 100 motile, freely moving, non-agglutinated spermatozoa and accepted at least 6/100 as a positive reaction, whereas Shulman et al (1975) related the number of spermatozoa involved in the agglutinates to the total number of cells (agglutinated and non-agglutinated) and required that at least 10% of the spermatozoa are involved in the agglutinates.

Several investigation have been aware of the risk of non-specific agglutination of spermatozoa around leucocytes, cellular debris and dead sperm cells (Schwimer et al, 1967a; Boettcher and Hay, 1969; Israelstam, 1969; Glass and Vaidya, 1970; Jones et al, 1973a; Petrunia et al, 1976). The hazard of false positive reactions has been reduced in some studies by including known negative control sera in each experiment (Israelstam, 1969; Glass and Vaidya, 1970; Vaidya and Glass, 1971; Kolodny et al, 1971).

Although all sexually active women are exposed to a relatively extensive load of sperm antigens, few seems to develop humoral immunity against spermatozoal surface antigens. Hancock (1978) has presented several possible explanations against spermatozoa in women.

1. Spermatozoa are flushed down the female genital tract by secretions (Austin, 1976), reducing the number that gain access to the immune system.
2. Certain seminal plasma components seem to have an immune-suppressing effect (Lord et al, 1977).
3. Extensive phagocytosis of spermatozoa in the female genital tract (Moyer et al, 1970; Austin, 1976) may degrade the spermatozoa, reducing their immunogenicity.

The limited number of immune reactions in women exposed to spermatozoa may also be due to insufficient amount of antigen resulting in low non-tolerance rather than in antibody production.

The higher incidence observed by various workers could be explained by various reasons. It seems likely that genital infections or epithelial lesions of various kinds may act as

triggering factors for local and subsequently systemic immune reactions against spermatozoa.

A significantly higher frequency of sperm-agglutinating activity in sera has been found in prostitutes compared with normal fertile women (Schwimmer et al, 1967b; Kolodny et al, 1971). This may reflect either a more extensive exposure to sperm antigens or a higher occurrence of genital infections.

Incidence of positive sperm agglutination in cases of primary infertility was 11.62%, while in secondary infertility 10% in sera (Table VII). Incidence shown by various workers in primary and secondary infertility was shown in Table XIX.

Table XIX

Incidence of sperm agglutinating activity in serum of primary and secondary infertility.

Name of workers	Primary infertility %	Secondary infertility %
1. Ansbacher et al (1971)	20.4	6.0
2. Hingorani et al (1978)	13.8	88.5
3. Nanda and Panigrahi (1978)	16.8	6.6
4. Upadhyay et al (1979)	28.6	16.0
5. Mishra and Das (1981)	10.0	-

Our present series is very small to give any conclusion regarding the incidence of sperm agglutinating activity in primary and secondary infertility.

To establish the sensitivity of the test sperm agglutination test was also observed in diluted sera and agglutination activity was also recorded at different hours. In cervical mucus dilution was not done because of little amount of cervical mucus, sera had been tested in dilution by several investigators because of risk of non-specific reaction with the undiluted sera (Shulman, 1975).

(ii) Sperm agglutinating activity in cervical mucus of study group -

In the present series sperm agglutinating activity in cervical mucus was present in 27.29% of women having unexplained infertility (Table XI).

Parish et al (1966) have demonstrated antisperm antibodies in 3 out of 11 patients (27.27%). Parish and Ward (1968) have demonstrated antisperm antibodies in 3 out of 48 cases (6.25%). Eyquen and D Almeida (1973) demonstrated antisperm antibodies in cervix mucus in 20% cases. Coelingh et al (1974)

demonstrated positive result in 3 out of 13 patients (23.77%). They supported the concept of local production of antibodies.

Shulman et al (1975) demonstrated the incidence of 15-30% sperm agglutinating activity in cervical mucus of women having unexplained infertility by tube slide agglutination techniques.

Sudo et al (1977) demonstrated the sperm agglutinating activity in 2.7% of cases. Ingerslev and Hjort (1979) demonstrated the antisperm antibodies in 3.8% of cases and Moghissi et al (1980) in 16.3% of cases having unexplained infertility. Mukerjee et al (1984) reported the incidence of 64% positive sperm agglutination in cervical mucus by MIS test. Misra et al (1984) reported the positive sperm agglutination in cervical mucus in 8% of cases having unexplained infertility (Table XX).

Table XX

Incidence of antisperm antibodies in cervical mucus reported by various workers.

Name of workers	Unexplained infertility positive cases %
1. Parish et al (1966)	27.27
2. Parish and Ward (1968)	6.225
3. Eyquem & D Almeida (1973)	20.0
4. Coeling et al (1974)	23.77
5. Shulman et al (1975)	15 - 30%
6. Sudo et al (1977)	2.7
7. Ingerslev (1979)	5.8
8. Moghissi et al (1980)	16.3
9. Mukerjee et al (1984)	64.0
10. Misra et al (1984)	8.0

Our results are almost consistent with the results of Parish et al (1966); Coeling et al (1974); Shulman et al (1975), while they are higher than Parish and Ward (1968); Sudo et al (1977); Ingerslev (1979); Misra et al (1984). Mukerjee et al (1984) gave a very high incidence of positive sperm agglutinating activity in cervical mucus.

The variation in results could be explained on the basis of different techniques used to detect the sperm antibodies and presence of concurrent infections, because several bacterial species are spermicidal in vitro (Mathews & Buxton, 1951; Buxton et al, 1954; Kay et al 1954; Teague et al, 1971), and production of antibodies in cervical mucus following infection has been demonstrated by various workers (Kerr, 1955; Bell and Wolf, 1967; Omran and Hulka, 1971; Wilkie et al, 1972; Corbeil et al, 1974b; Yang & Schumacher, 1979).

The cervix seems to be the probable origin of local and systemic immune reactions against spermatozoa : (i) The cervix appear to contain a local immune system and the immune potential is higher than in any other segment of the female genital tract, as evaluated by immuno-histological investigation and experimental immunization studies.

(ii) The cervix is exposed to a significantly greater number of spermatozoa and accordingly to a more extensive antigenic load than higher part of the female genital tract (Ahlgren, 1969).

A significantly rising incidence of sperm agglutination in cervical mucus was observed with

increasing duration of infertility (Table XII), justifying the criteria that cervix is exposed to the greater number of antigenic spermatozoa and also for longer period in these cases.

Incidentally same pattern of rising incidence of sperm agglutinating activity in serum was also observed with increasing duration of infertility (Table VIII). Mishra et al (1981), Mukerjee et al (1984), Misra et al (1984) also observed that presence of antibodies had definite correlation with duration of infertility. With increasing duration of infertility, higher prevalence of positive cases have been demonstrated. However, no significant difference was observed in the incidence of positive cervical mucus result in primary and secondary infertility (Table XI).

Correlation between sperm agglutinating activity in serum and cervical mucus -

(i) Agglutinating activity in cervical mucus in cases with positive sera -

Table XV indicates, majority of cases with positive serum showed positive result in the cervical mucus. Sperm antibodies in serum probably appears as a

consequence of sperm antigen being exposed to the immune system in the genital tract. It was therefore reasonable to believe that antibodies should be present concomitantly in serum and genital secretions. Shulman (1974) studied a total of 56 cervical mucus sample from 38 women who had positive antibodies in serum. They observed that infertile women with antibody positive serum had positive cervical mucus in 82% by F.D. test and 67% by Kibrick test.

However, several reports have demonstrated that only 40-60% of women with sperm agglutinating antibodies in serum have detectable antibodies in cervical mucus (Shulman and Guerrere, 1978; Mughissi et al, 1980; Ingerslev, 1980).

It is not likely that women with positive serum but negative cervical mucus, are immunized in higher part of genital tract where immune potential is low. It is also unlikely that few spermatozoa entering the peritoneal cavity during mid cycle (Ahlgren, 1969) constitutes an insufficient stimulus to stimulate a systemic immune reactions.

The apparently shorter persistence of local immune reactions compared to systemic immune reactions

(Ogra and Karzon, 1969; Rossen et al, 1970; Couch et al, 1971; O'Reilly et al, 1976), is more obvious explanation for negative test in cervical mucus from women with circulating sperm antibodies.

Another explanation could be that, the spontaneous cure of "triggering" condition like infection may lead to cessation of contact between sperm antigen and immune system, despite continued sexual activity. The local antibodies may subsequently disappeared, whereas, the antibodies in serum probably persist for much longer period.

Ahgren (1969) suggested that few spermatozoa reaching the peritoneal cavity during the mid cycle may maintain systemic immune reactions once this has been established even in absence of local reaction.

(ii) Agglutinating activity in sera in cases with positive cervical mucus -

Table XVI shows that out of 26 women with positive sperm agglutinating activity in cervical mucus, 9 women had concurrent sperm agglutinating activity in serum (34.61%).

According to Sudo et al (1977) and Donadro et al (1978) antibodies against sperm may

only be found at local sites without showing their presence in systemic circulation.

Ingerslev (1980) investigated cervical mucus from 21 infertile women with sperm agglutinating antibodies in serum by applying the tray agglutination technique to bromaline treated cervical mucus, 8 of the women (38%) had concurrent sperm agglutinating antibodies in ovulatory cervical mucus.

Moghissi et al (1980) found negative serum in 22 of the 28 women with sperm agglutinating activity in cervical mucus with an incidence of only 21.4% positive result. Explanation for this could be that they tested the cervical mucus by more sensitive tray agglutination technique.

Sperm antibodies in serum probably appears as a consequence of sperm antigens being exposed to the immune systems in the genital tract. It was therefore reasonable to believe that antibodies should be present concomittently in serum and genital secretions.

Correlation of antisperm antibodies with post coital test -

It was observed that poor, sluggish and no motility of sperm was associated with positive

sperm agglutination in cervical mucus. This suggest that sperm loose their power of motility due to sperm agglutinating antibodies in the cervical mucus. Therefore a poor post coital test could be taken as an indication for the study of antisperm antibodies.

Parish and Ward (1968) observed the same result and assigned the same significance to the study of antisperm antibodies in case with poor post coital test. However, Wall et al (1975) stated that poor result on post coital test were due to some factor, other than spermatozoal antibodies. Sinha et al (1977) suggested local immune reaction as a cause of poor post coital test and infertility.

Several subsequent investigations by Kremer and co-workers reported the relationship between the presence of sperm agglutinating antibodies in cervical mucus and poor post coital with poor sperm penetration test (Kremer and Jager, 1977; Kremer et al, 1977; Kremer et al, 1978 a; Hansen & Hjert, 1979; Moghissi et al, 1980 ; and Ingerslev, 1980). They demonstrated that sperm agglutinating antibodies in cervical mucus or in seminal plasmas may cause infertility by impeding sperm penetration in cervical mucus.

Kremer and co-workers (1977 & 1980) have described 7 women with sperm agglutination antibodies in cervical mucus, all had poor post coital test.

Ingerslev (1980) observed lower titers and less affected sperm motility in cervical mucus. The apparent discrepancy between their result probably due to different selection of patients.

In most cases with a so called unexplained poor post coital test, occurring in the presence of optimal ovulatory cervical mucus and normal sperm sample, the cause is probably antibodies against spermatozoa. This evidence is further supported by Kremer et al (1978) and Moghissi et al (1980). They demonstrated that the number of good or excellent post coital test declined notably in the presence of sperm antibodies in cervical mucus in couples with normal cervical mucus and normal sperm sample. A negative or poor post coital test was observed in cervical mucus from 12 out of 18 women (66%) with sperm agglutinating antibodies in cervical mucus and in 9 and 11 (82%) with sperm immobilizing activity in the cervical secretions (Moghissi et al, 1980).

Regarding the pattern of agglutination there were either head to head, tail to tail and mixed type or combination depending upon the site of antigen present over the surface of spermatozoa. Glass and Vaidya (1971) have shown head to head pattern, as the most common pattern. Shulman (1973) showed tail to tail type to be most frequent. Mishra and Dass (1981) have shown all the three type of agglutination in their observation. In our study, head to head pattern was observed more commonly in cervical mucus as well as in serum (Table X & XIV).

From all these results, it appears that agglutination of any part of sperm may be taken as reliable index for presence of sperm agglutinating antibodies.

All the cases with positive sperm agglutinating antibodies were advised treatment with a view to destroy the antibodies and to prevent the further immunization. Women with positive sperm agglutination test in sera and cervical mucus advised condom therapy along with Corticosteroids for 6 wk to 3 months. After stoppage of steroid therapy, patients were kept on estrogen therapy. Tab. ethinyl estradiol 50 µg O.D. from 5th to 25th day of period for 3 months.

The infertile couples having antisperm antibodies were kept on condom therapy from 2 - 12 months interval by Franklin and Dukes (1964), Schwimmer et al (1967), Glass & Vaidya (1970), Kolodny et al (1971), Mukerjee et al (1973), Jones et al (1976) and Srivannaboon et al (1982). They reported that significant number of women become pregnant subsequent to the resumption of unrestricted coitus. Ingerslev (1980) reported the significance decrease titer of sperm antibodies in cervical mucus with oestrogen therapy.

In the present series, cases are being followed but none of them have returned with pregnancy.

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CONCLUSION

### C O N C L U S I O N

The present study was undertaken to evaluate the significance of antibodies against spermatozoal surface membrane antigens in female infertility.

A total of 106 women were studied. The study group comprised of 96 women having unexplained infertility, while 10 women with known fertility were taken as control group.

Out of 96 women, 11 (11.46%) showed the positive result in serum, while 26 women (27.28%) showed positive result in cervical mucus. Nine women showed sperm agglutinating activity in serum and cervical mucus both, 2 women had sperm agglutinating activity in sera only, while 17 women showed positive agglutination in cervical mucus only.

In brief it was concluded that -

1. Antisperm antibodies in serum or in cervical mucus had significant role in female infertility.
2. Cervix is definitely a local site for antibody production and a possible factor for infertility in women of unexplained etiology.

3. There is significant association between local and systemic immune reactions against spermatozoa in female infertility.
4. Local immune reaction persist for shorter time than systemic immune reactions, therefore the antibodies in cervical mucus may subsequently disappear whereas the antibodies in serum persist for much longer period.
5. Presence of antibodies had definite correlation with duration of infertility. Increasing duration of infertility had higher prevalence of positive cases.
6. Poor P.C.T. is a significant criteria for evaluating the immunological cause for infertility and significantly associated with sperm antibodies in cervical mucus.

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B I B L I O G R A P H Y

B I B L I O G R A P H Y

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ABBR E V I A T I O N S

F.D. : Franklin and Dukes

P.C.T. : Post Coital Test

Cx. mucus : Cervical mucus.

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S U M M A R Y

## S U M M A R Y

The present study was undertaken to evaluate the significance of antibodies against spermatozoal surface-membrane antigens in female infertility.

"Comparison between sperm agglutinating activity in serum and cervical mucus of infertile women" was carried out in the Department of Obstetrics and Gynaecology in collaboration with the Department of Pathology, M.L.B. Medical College, Jhansi.

A total of 106 women were studied for the incidence of sperm agglutinating activity in serum and cervical mucus. Ten women of known fertility were taken as control group while, 96 women having unexplained infertility were taken as study group.

All the women were screened on the basis of clinical history, examination and investigations. The infertile women were further sub-grouped into primary infertility including 86 women and secondary infertility including 10 women.

The age of the women ranged from 19-40 years and maximum number of cases (36.47%) were between the age of 21-30 years. The duration of infertility ranged from 2 - 20 years. Maximum number of cases (52.08%) were having infertility of 6 - 10 years duration.

The sperm agglutination test of Franklin and Dukes (1964a) was used in a slightly modified way for the presence of sperm agglutinating activity in serum, Microscale test of cervical mucus extract was used for sperm agglutinating activity in the cervical mucus.

Out of 96 women, 11 (11.46%) having unexplained infertility showed positive sperm agglutination test in serum while 26 women (27.29%) showed positive test in cervical mucus. None of the control cases showed positive result either in sera or in cervical mucus.

Out of 11 women with positive sperm agglutinating activity in sera, 9 women showed agglutinating activity in cervical mucus (81.8%) while 2 women showed agglutinating activity in sera only (18.2%).

Out of 26 women with positive sperm agglutinating activity in cervical mucus, 9 women had agglutinating activity in serum also (34.61%), while 17 women had sperm agglutinating activity in cervical mucus only (73.07%).

There were no significant difference in sperm agglutinating activity in serum and cervical mucus of both the groups; primary and secondary infertility.

Women having positive sperm agglutination test were kept on condom therapy along with corticosteroids for 6 weeks to 3 months. After stoppage of corticosteroid treatment, women were kept on oestrogen therapy. Till now none of them have returned with pregnancy. It is yet to be seen how far this treatment would be helpful in such cases. As the number of cases in our study was small, no conclusive opinion could possibly be drawn at present.

On analysis of the result, it is apparent that

- (i) Cervix is definitely a local site for antibody production and possibly a factor for infertility in women having unexplained infertility.

- (ii) Poor Post Coital test definitely suggests a local immune reaction and signifies the study of anti-sperm antibodies at the local site, i.e. cervix mucus.
- (iii) Antibodies against spermatosoma may only be found at local site without showing their presence in systemic circulation and vice-versa.

